REMARKS

Applicant first wishes to thank the Examiner for the helpful telephonic interview on May 20, 2009. Pursuit to that interview, Applicant presents the foregoing amendments and following remarks.

Claim Status

Claim 1 has been amended to characterize that the fluorescence quenching leaving group contains a sulfur atom bonded to three oxygen atoms and a carbon chain. Support for such amendment is clearly found in paragraph [0035] and Figs. 2-3 of the original application.

Additionally, claim 1 has been amended to include three more exemplary fluorescence quenching leaving groups as described in paragraph [0022] as well as illustrated in Fig. 2 of the original application.

Claims 2-4 have been cancelled without prejudice.

Previously withdrawn claims 17, 21 and 25 have been amended to refer to conditions used for intramolecular or intermolecular chemical ligations or nucleic acid hybridizations of the present invention, wherein no exogenous enzymes are added. Support for such amendments is clearly found in paragraphs [0019], [0067], and the Abstract of the original application.

Previously withdrawn claims 18, 22 and 38 have been amended to refer the method steps back to the preamble of the claims. Support for such amendments is clearly found in paragraphs [0031], [0042], the Abstract as well as Examples 1-2 of the original application.

New claims 51-52 have been added, directed to a probe pair. Support for these new claims is clearly found in paragraphs [0034], [0035] and [0069] as well as in Figs. 2-3 of the original application.

Applicant respectfully submits that the foregoing amendments do not introduce any new matter to the application as originally filed. With the foregoing amendments, claims 1, 5-14 and 16-27 and 29-52 are currently pending, among which claims 16-27 and 29-39 have been previously withdrawn by the Examiner as being drawn to non-elected species and inventions. Applicant notes that the previously withdrawn claims are being requested to be rejoined with the elected product claims for further examination upon the allowance of the product claims. *See* MPEP §821.04.

Additionally, newly added claims 51-52, directed to a probe pair comprising an electrophile probe and a nucleophile probe, are patentably related to the previously elected product claims 1 and 5-14. There is a common technical feature that ties these claims together, that is, a fluorophore compound (i.e., an electrophile probe) comprising a fluorophore group and a fluorescence quenching leaving group, the fluorescence quenching leaving group containing a sulfur atom bonded to three oxygen atoms and a carbon chain, and selected from the list of examples identified therein. As such, searches for new claims 51-52 and previously elected product claims would be co-extensive and would not be unduly burdensome to the Examiner. Accordingly, Applicant respectfully requests that new claims 51-52 be joined with claims 1 and 5-14 for further examination.

Claim Interpretation

The Examiner states that the term "fluorescence quenching leaving group" is not defined. In particular, the Examiner states that Applicant did not define what it means for the

fluorescence to be quenched, and that the term "leaving group" has not been defined. As such, the term "fluorescence quenching leaving group" is interpreted as any fluorescence quenching group. In response, Applicant respectfully disagrees for the reasons stated below.

As repeatedly argued previously, the instant specification clearly sets the metes and bounds of the terms "fluorescence quenching leaving group" and "leaving group". Not all fluorescence quenching groups are considered as fluorescence quenching leaving groups according to the context of the present application.

In particular, the instant specification provides the following definition to the term "leaving groups" in paragraph [0035]:

> Examples of quenching leaving groups are shown in Figure 2 (note that in the figure they are attached to the 5' carbon of a nucleoside, but they can be attached to any atom that is reactive with a nucleophile. The leaving groups in the figure include the sulfur and the three oxygen atoms attached to it, as well as the carbon chain attached to sulfur. Leaving groups as in general are defined by (a) their ability to activate an atom (to which they are attached) for attack by a nucleophile group and (b) to leave (either simultaneously or subsequently) when the nucleophile does attack. (Emphasis added) *Id.*

One of ordinary skill in the art of organic chemistry would understand that in the context of the present invention the term "leaving group" means a compound having a structure as such that it would promote nucleophilic attack and that it would leave when the nucleophile does attack. That is, the "leaving group" of the present invention has a structure that is reactive with a nucleophile. Nevertheless, Applicant has amended the instant claims to recite that the fluorescence quenching leaving group contains a specific structure of a sulfur atom bonded to three oxygen atoms and a carbon chain. The above description

provides direct support for the present amendment to claim 1 with respect to the structural feature of the fluorescence quenching leaving group.

As to the term "quenching", the instant specification describes that nucleophilic attack on the quenched DNA causes release of the quencher group, which results in a ligated molecule that is now fluorescent due to the absence of the quencher group (*see*, paragraph [0019]), and that upon ligation with another molecule in intermolecular fashion, or with itself in intramolecular fashion, the quenching leaving group is displaced and the fluorophore is no longer quenched (*see*, paragraph [0031]). In addition, examples are given regarding quenched fluorescence. Example 2 of the instant specification describes that when beads containing a 7mer MUT probe autoligate a 13mer quenched electrophile probe to themselves, in the presence of the correct target DNA, the beads would become fluorescent, as the dabsylate group was lost and the nearby fluorescein label lost quenching.

The above descriptions and example clearly indicate that quenched fluorescence in the context of the present invention involves a quenched electrophile probe, which is a unimolecular entity with both a fluorophore group (e.g., fluorescein) and a fluorescence quenching leaving group (e.g., dabsylate group) located close to each other thereon.

In addition, the leaving group itself is also a quencher in the context of the present application, such that when the bond is broken and the leaving group leaves, the fluorescence of the remaining molecule increases.

Furthermore, the instant specification provides multiple examples of fluorescence quenching leaving group (*see*, paragraphs [0041] and [0042].)

Applicant contends that the above descriptions and examples from the instant specification define the term "fluorescence quenching leaving group" through the term usage in the context of the specification. As implicated, the fluorescence quenching leaving group

in the context of the present invention is not any fluorescence quenching group; rather, it is a group that is both a quencher and a leaving group.

The Court of Appeals for the Federal Circuit has ruled that the specification is the single best guide to the meaning of a disputed term and that even when guidance is not provided in explicit definitional format, the specification may define claim terms by implication such that the meaning may be found in or ascertained by a reading of the patent documents. See *Phillips v. AWH Corp.*, 415 F.3d 1303, 75 USPQ2d 1321 (Fed. Cir. 2005) (*en banc*); *Vitronics Corp. v. Conceptronic Inc.*, 90 F.3d 1576, 39 USPQ2d 1573, 1577 (Fed. Cir. 1996); and *Novartis Pharms. Corp. v. Abbott Labs.*, 375 F.3d 1328, 1334-35 (Fed. Cir. 2004) (copies previously provided).

At page 3 of the present Office Action, the Examiner states the following:

> The term "leaving group" has not been defined. Further, this is a functional, not a structural limitation, since for every chemical bond there is a nucleophile and a set of conditions under which the bond can be broken. Finally, "leaving" simply means getting separated from the probes, which can be achieved by enzymatic cleavage, for example. *Id.*

Applicant respectfully disagrees with the Examiner's above statement. First, the term "leaving group" is used instead of the word "leaving". In fact, the term "leaving group" is used 69 times throughout the original application as filed. There is not even once that only the word "leaving" is used throughout the original application. The term "leaving group", which is a compound noun, has very specific chemical meaning when combined with "nucleophile". As discussed above, one of ordinary skill in the art of organic chemistry would understand that in the context of the present invention the term "leaving group" means a compound having a structure as such that it would promote nucleophilic attack and that it

would leave when the nucleophile does attack. That is, the "leaving group" of the present invention has a structure that is reactive with a nucleophile.

One of ordinary skill in the art of organic chemistry would also know that "leaving group" and "nucleophile" are part of the terminology of the "nucleophilic displacement" reaction (also called "nucleophilic substitution" or "S_N2 reaction"), which is a very common chemical reaction. A nucleophile reacts at an electrophilic atom, and a leaving group departs the atom at the same time. The following are quoted from two common organic chemistry textbooks with emphasis added on the term "leaving group". Copies of the related pages of the textbooks are enclosed herein for the Examiner's consideration.

- McMurry, *Organic Chemistry*, 2nd Ed., p. 341-342 states:

  The Leaving Group
  A further variable that can strongly affect the S_N2 reaction is the nature of the species expelled by the attacking nucleophile – the **leaving group**. Since the leaving group is expelled with a negative charge in most S_N2 reactions, we might expect the best **leaving groups** to be those that best stabilize the negative charge.

- Vollhardt and Schore, *Organic Chemistry*, 5th Ed. p. 231 states:

  ...nucleophilic substitution occurs only when the group being displaced, X, is readily able to depart, taking with it the electron pair of the C-X bond...the relative rate at which it can be displaced... can be correlated with its capacity to accommodate a negative charge.

  ...

  Halides are not the only groups that can be displaced by nucleophiles in S_N2 reactions. Other examples of good **leaving groups** are sulfur derivatives and...various sulfonate ions.

In the present invention, the fluorescence quenching leaving group (e.g., a dabsylate group) has very special mechanisms designed into it to stabilize the negative charge, that is,

the negative charge is shared on three oxygen atoms for stabilization (*see* paragraph [0035] and Figs. 2-3 of the original application).

Additionally, the Examiner's allegation that every chemical bond can be cleaved by a nucleophile is baseless. In fact, the large majority of chemical bonds cannot be cleaved by a nucleophile, especially under the condition of a mild temperature range of 0-100 degrees (not 1000 degrees) in water. Only very specialized molecular structures can act as leaving groups under these mild conditions with only iodide, bromide, sometimes chloride, and a few specialized sulfonates listed in common textbooks. *See* Table 11.5 in McMurry, *Organic Chemistry*, 2nd Ed., p. 342.

Furthermore, the Examiner states that the terms "about 2 fold", "about 100 fold" and "about 1000 fold" are not defined. Applicant first notes that the terms "about 2 fold", "about 100 fold" and "about 1000 fold" are not recited in the currently pending claims.

Additionally, Applicant respectfully submits that one of ordinary skill in the art would readily understand what the terms mean, especially with the definition given in the instant specification that the efficiency of quenching is the unquenched fluorescence with the fluorescence quenching group absent divided by the quenched fluorescence with the fluorescence quenching group present. However, in order to further the examination, Applicant has cancelled, without prejudice, claims 2-4 that are related to quenching efficiency.

Claim Rejection – 35 USC §112, Second Paragraph

Claims 2-4 stand rejected under 35 USC §112, second paragraph, for allegedly being indefinite. In particular, the Examiner alleges that the term "at least x fold" is not defined.

In response, Applicant has cancelled claims 2-4 without prejudice. As such, this rejection is moot.

Claim Rejection – 35 USC §102

Claims 1-7, 9-12, 14 and 50 stand rejected under 35 USC §102 as allegedly being anticipated by Aoyagi et al. (U.S. Patent No. 5,952,202; "**Aoyagi**"). In response, Applicant respectfully traverses this rejection.

As presently amended, the instant claims are directed to a composition comprising a fluorophore compound, which comprises a fluorophore group and a fluorescence quenching leaving group, wherein the fluorescence quenching leaving group contains a sulfur atom bonded to three oxygen atoms and a carbon chain, and is selected from the list of examples identified therein.

Aoyagi discloses a probe that is quenched and that can be cleaved by an enzyme, causing the quencher to be separated from the fluorescent label. **Aoyagi**'s probe does not contain any leaving groups as defined in the instant specification and as understood by one of ordinary skill in the art, nor does **Aoyagi** mention the word "leaving group".

Although it teaches dabsyl as a quencher, **Aoyagi** does not teach or suggest the same kind of dabsyl group as that of the present invention. In particular, **Aoyagi**'s dabsyl, as illustrated in Figure 3 thereof, contains a sulfur atom and two oxygen atoms and a carbon chain attached to the sulfur atom. In contrast, the instantly claimed fluorescence quenching leaving group; e.g., a dabsylate group, comprises a sulfur atom and three oxygen atoms and a carbon chain attached to the sulfur atom. As discussed above, the additional oxygen atoms contained in the fluorescence quenching leaving group of the present invention share

negative charge and further stabilize the negative charge as required for "leaving group" as defined by the instant specification and as understood by one of ordinary skill in the art.

Additionally, **Aoyagi**'s dabsyl is attached to a nitrogen atom, whereas Applicant's dabsyl group is attached to an oxygen terminal atom. As mentioned in paragraph [0042] of the original application (quoted below), dabsyl has previously been attached to amines, wherein it cannot act as a leaving group. **Aoyagi** is one of the previous reports on dabsyl being a fluorescent quencher, but not a leaving group.

> [0042] Dabsyl has long been used as a fluorescence quencher. However it has never before been used as a leaving group. It has previously been attached to amines, where it cannot act as a leaving group. It is the ability to act as a leaving group that allows embodiments of this invention to be successful: it causes the nucleophile to attack, and then it leaves, causing fluorescence to increase. *Id.*

That is, **Aoyagi**'s dabsyl has a chemically distinct structure from the dabsyl leaving group of the present invention. Although recognized as a useful quencher, **Aoyagi**'s dabsyl cannot be a "leaving group" as defined in the instant specification and as understood by one of ordinary skill in the art. No nucleophilic displacement reaction can occur. In contrast, the dabsyl leaving group of the present invention contains a terminal atom of oxygen which provides an additional oxygen atom for stabilizing the negative charge. Such stabilizing structure renders the dabsyl group into an active "leaving group" as described in the instant specification and as understood by one of ordinary skill in the art, making nucleophilic displacement a rapid and facile reaction in water at physiological pH and at room or physiological temperature (i.e., mild conditions).

At page 5 of the present Office Action, the Examiner states that **Aoyagi** inherently teaches nucleophilic groups since they work with DNA/RNA which has NH and OH groups,

which can act as nucleophiles. However, Applicant submits that **Aoyagi** does not use these nucleophiles to make the bond cleave or make the quencher leave, nor does **Aoyagi** claim or use the word "nucleophile". Instead, **Aoyagi** uses an enzyme to cleave the probes. That is, the cleavage reaction in **Aoyagi** requires an enzyme.

In fact, the nucleophiles of **Aoyagi** cannot and do not cause the probes to be cleaved through nucleophilic attack because of the reasons stated below.

First, the NH and OH groups are extremely weak nucleophiles compared to phosphorothioate as shown in Table 6-7 of Vollhardt and Schore, *Organic Chemistry*, 5th Ed. (Copies of the related pages are enclosed herein.) It is taught therein that anionic sulfur nucleophiles similar to the phosphorothioate nucleophile of the present invention (shown as HS- in the table) are 100 million times better nucleophiles than alcohols (demonstrated by CH_3OH in the table). Sulfur anion nucleophiles are more than 300 times better nucleophiles than aliphatic amines (demonstrated by NH_3 in the table). Furthermore, the NH_2 groups in DNA are arylamines, which are significantly poorer nucleophiles than aliphatic amines because they are much less basic.

In addition, McMurry, *Organic Chemistry*, 2nd Ed., states at p. 935: "[t]he base strength of arylamines is generally less than that of aliphatic amines, however. Thus, methylammonium ion has $pK_a = 10.64$, whereas anilinium ion has $pK_a = 4.63$." (Copies of the related pages are enclosed herein.) That is, the alcohols and amine groups in DNA are thousands and up to millions times poorer nucleophiles than the phosphorothioate of the present invention. This renders NH and OH groups ineffective as nucleophiles for the intended purpose.

Secondly, there is no "leaving group" on **Aoyagi**'s molecule as defined by the instant specification and as understood by one of ordinary skill in the art. In **Aoyagi**, the probes are

stable and will not be cleaved until the exonuclease enzyme is added. Although the quencher in **Aoyagi** is ultimately separated from the fluorescent label due to the action of an enzyme, there is no known nucleophile that can cause this separation with **Aoyagi**'s probes under any chemically reasonable conditions in water without added enzymes.

In light of the foregoing amendments and remarks, Applicant respectfully submits that **Aoyagi** does not anticipate the present invention as claimed and therefore, request that the novelty rejection over **Aoyagi** be withdrawn.

Claim Rejection – 35 USC §103(a)

Claim 8 stand rejected under 35 USC §103(a) as allegedly being unpatentable over **Aoyagi** and Lee et al. (U.S. Patent No. 6,348,596; "**Lee**"). In response, Applicant respectfully traverses this rejection.

As discussed above, **Aoyagi** teaches a dabsyl with a chemically distinct structure from the dabsyl leaving group of the present invention. **Aoyagi** does not teach or suggest a fluorescent quenching leaving group of the present invention.

Instant claim 8, dependent from instant claim 1, is directed to a double-stranded nucleic acid composition comprising a fluorophore compound, the fluorophore compound comprising a fluorophore group and a fluorescence quenching leaving group, wherein the fluorescence quenching leaving group contains a sulfur atom bonded to three oxygen atoms and a carbon chain for stabilizing the negative charge for cleavage by nucleophilic attack, and is selected from the list of examples identified therein.

Lee discloses non-fluorescent asymmetric cyanine dye compounds useful for quenching reporter dyes. **Lee** does not teach or suggest a composition comprising a fluorophore compound, the fluorophore compound comprising a fluorophore group and a

fluorescence quenching leaving group, wherein the fluorescence quenching leaving group contains a sulfur atom bonded to three oxygen atoms and a carbon chain for stabilizing the negative charge for cleavage by nucleophilic attack. That is, **Lee** does not compensate the above-discussed deficiency of **Aoyagi**.

The Examiner states that although **Aoyagi** does not teach double-stranded probes, **Lee** teaches fluorescently-labeled energy-transfer probes which can be either single-stranded or double-stranded, and that it would be obvious to one of ordinary skill in the art at the time of the invention to have used alternative label configurations suggested by **Lee** in the fluorophore-quencher detection system of **Aoyagi**. In response, Applicant respectfully submits that even if the alleged obviousness existed, one of ordinary skill in the art would have arrived at a double-stranded probe comprising dabsyl as a quencher with a structure of a sulfur atom and two oxygen atoms and a carbon chain attached to the sulfur atom. Such probe would still be different from the claimed composition of the present invention as discussed above.

In light of the foregoing amendments and remarks, Applicant respectfully submits that **Aoyagi** and **Lee**, alone or combined, do not render instant claim 8 obvious and therefore, request that the obviousness rejection over the two references be withdrawn.

Claim 13 stand rejected under 35 USC §103(a) as allegedly being unpatentable over **Aoyagi** and Mayrand et al. (U.S. Patent No. 5,691,146; "**Mayrand**"). In response, Applicant respectfully traverses this rejection.

As discussed above, **Aoyagi** teaches a dabsyl with a chemically distinct structure from the dabsyl leaving group of the present invention. **Aoyagi** does not teach or suggest a fluorescent quenching leaving group of the present invention.

Instant claim 13, dependent from instant claim 1, is directed to a fluorophore compound comprising a fluorophore group, a fluorescence quenching leaving group, and a phosphorothioate or a phosphoroselenoate as a nucleophilic group, wherein the fluorescence quenching leaving group contains a sulfur atom bonded to three oxygen atoms and a carbon chain for stabilizing the negative charge for cleavage by nucleophilic attack, and is selected from the list of examples identified therein.

Mayrand discloses an oligonucleotide probe including a fluorescent molecule attached to a first end of the oligonucleotide and a quencher molecule attached to the opposite end of the oligonucleotide. **Mayrand** also teaches blocking the 3' end of the probe to enzyme activity with modified internucleotide linkages including phosphorothioate groups. However, **Mayrand** does not teach the use of the phosphorothioate as a nucleophile, nor does the phosphorothioate cause cleavage of the quencher from the fluorophore. In fact, the phosphorothioate cannot cleave the probe because there is no "leaving group" on the probe as defined by the instant specification and as understood by one of ordinary skill in the art. That is, **Mayrand** does not compensate the above-discussed deficiency of **Aoyagi**.

The Examiner states that although **Aoyagi** does not teach phosphorothioate groups, **Mayrand** teaches using phosphorothioate groups to block the 3' end of the probe to enzyme activity and as such, it would be obvious to one of ordinary skill in the art at the time of the invention to have used alternative means for blocking the 3' terminus of the probe suggested by **Mayrand** in the method of **Aoyagi**. In response, Applicant respectfully submits that even if the alleged obviousness existed, one of ordinary skill in the art would have arrived at a probe comprising dabsyl as a quencher as well as phosphorothioate as a blocking agent, wherein the dabsyl has a structure of a sulfur atom and two oxygen atoms and a carbon chain

attached to the sulfur atom. Such probe would still be different from the claimed composition of the present invention as discussed above.

In light of the foregoing amendments and remarks, Applicant respectfully submits that **Aoyagi** and **Mayrand**, alone or combined, do not render instant claim 13 obvious and therefore, request that the obviousness rejection over the two references be withdrawn.

Allowable Subject Matter

Applicant notes that kit claims 40-49 remain allowed in the present Office Action as stated by the Examiner at page 7 thereof. Applicant appreciates the Examiner's statement of allowability of claims 40-49; however, it is believed that the forgoing amendments and remarks would place the other product claims in condition for allowance as well. As such, Applicant respectfully requests that the Examiner further the examination on the other product claims.

Rejoining of Non-Elected Groups and Species

Upon the allowance of product claims of Group I, *i.e.*, claims 1, 5-14 and 50 as presently amended, Applicant respectfully requests that the method claims of Groups II and III, *i.e.*, claims 17-27 and 29-39, be rejoined for further examination. *See* MPEP §821.04. As previously amended, the withdrawn method claims incorporate all limitations of the product claims.

Also, Applicant respectfully requests that the claims directed to the non-elected species be rejoined with the claims directed to the elected species for further examination if the Examiner finds the claims directed to the elected species allowable upon considering the foregoing amendments and remarks. *See*, MPEP 809.02(a).

This response is filed concurrently with a petition for a three-month extension of time. The Commissioner is authorized to deduct the extension fee of $555 from Howrey LLP Deposit Account No. 08-3038/12665.0024.NPUS01. Should any additional fees be required for any reasons relating to this document, the Commissioner is authorized to deduct such fees from the same deposit account.

Respectfully submitted,

/j. wendy davis/

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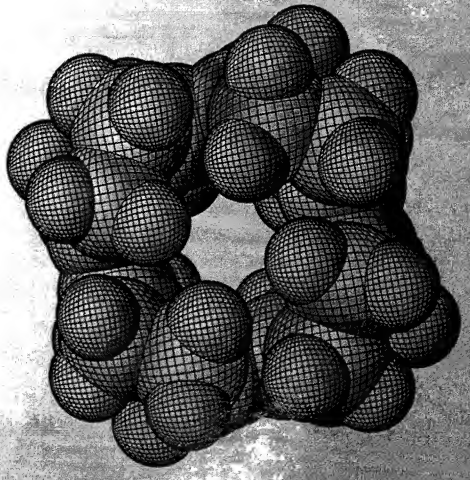
Date: May 21, 2009

SECOND EDITION

Organic Chemistry

John McMurry



SECOND EDITION

Organic Chemistry

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Cornell University

BROOKS/COLE PUBLISHING COMPANY
Pacific Grove, California

Table 11.4 Comparison of nucleophilicity and basicity for some oxygen nucleophiles; stronger bases are better nucleophiles

Nucleophile	*Relative nucleophilicity toward bromomethane*	*Conjugate acid*	pK_a
$CH_3CH_2\ddot{O}:^-$	25,000	CH_3CH_2OH	16
$H\ddot{O}:^-$	16,000	H_2O	15.7
	8,000		10
$CH_3CO_2:^-$	500	CH_3CO_2H	4.8
$H_2\ddot{O}:$	1	H_3O^+	−1.7

Nucleophilicity ↑ Basicity ↑

2. A second trend indicated in Table 11.3 is that nucleophilicity usually increases in going down a column of the periodic table. Thus $H\ddot{S}:^-$ is more nucleophilic than $H\ddot{O}:^-$, and the halide reactivity order is $:\ddot{I}:^- > :\ddot{B}r:^- > :\ddot{C}l:^-$.

PROBLEM

11.3 What products would you expect from reaction of 1-bromobutane with these reagents?

(a) NaI (b) KOH (c) H—C≡C—Na (d) NH_3

PROBLEM

11.4 The tertiary amine base quinuclidine reacts with CH_3I 50 times as fast as triethylamine. Can you suggest a reason for this difference?

$$R_3N: + CH_3I \longrightarrow R_3\overset{+}{N}—CH_3\ :\ddot{I}:^-$$

Quinuclidine

$(CH_3CH_2)_3N:$

Triethylamine

PROBLEM

11.5 Which reagent in each of the following pairs is more nucleophilic? Justify your choices.

(a) $(CH_3)_2\ddot{N}:^-$ and $(CH_3)_2\ddot{N}H$ (b) $(CH_3)_3B$ and $(CH_3)_3N:$

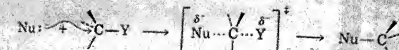
(c) $H_2\ddot{O}:$ and $H_2\ddot{S}:$

THE LEAVING GROUP

A further variable that can strongly affect the S_N2 reaction is the nature of the species expelled by the attacking nucleophile—the **leaving group**.

Since the leaving group is expelled with a negative charge in most S_N2 reactions, we might expect the best leaving groups to be those that best stabilize the negative charge. Furthermore, since anion stability is related to basicity, we can also say that the best leaving groups should be the weakest bases.

The reason that stable anions (weak bases) make good leaving groups can be understood by looking at the transition state. In the transition state for an S_N2 reaction, the charge is distributed over both the attacking nucleophile and the leaving group. The greater the extent of charge stabilization by the leaving group, the more stable the transition state and the more rapid the reaction.

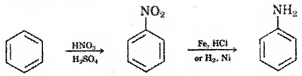


Transition state

Table 11.5 lists a variety of leaving groups in order of reactivity and shows their correlation with basicity. The weakest bases (anions derived from the strongest acids) are indeed the best leaving groups. The *p*-toluenesulfonate (tosylate) leaving group is the most easily displaced, although its basicity is out of line with others in the table for reasons that aren't well understood. Iodide and bromide ions are also excellent leaving groups, but chloride ion is much less effective.

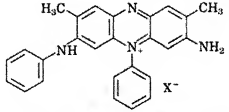
Table 11.5 Correlation of leaving-group ability with basicity; the anions of strong acids make good leaving groups in the S_N2 reaction

Leaving group	pK_a *of conjugate acid*	*Relative reactivity*
 Tosylate		
I^-	−6.5	60,000
Br^-	−9.5	30,000
Cl^-	−9	10,000
F^-	−7	200
$CH_3CO_2^-$	3.2	1
HO^-	15.8	



Benzene Nitrobenzene Aniline

Subsequent work showed that Perkin's original mauve was in fact not derived from aniline but from a toluidine (methylaniline) impurity in his starting material. Pure aniline yields a similar dye, however, which came to be marketed under the name *pseudomauveine.*



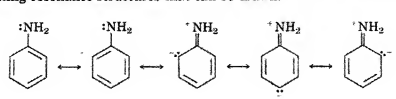
Perkin's mauve
(pseudomauveine has no methyl groups)

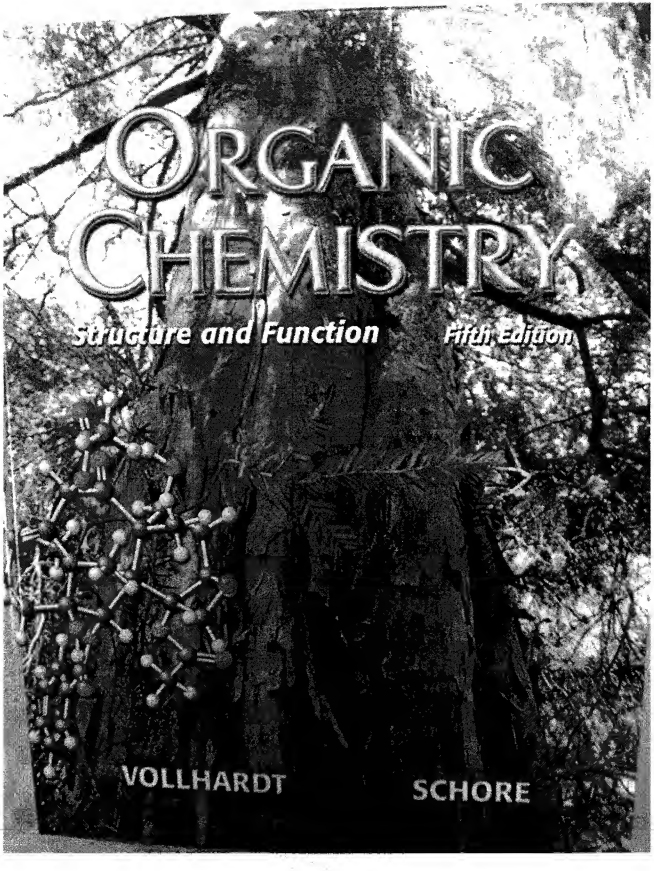
Today, dyestuff manufacture is a thriving and important part of the chemical industry, and many commonly used pigments are derived from aniline. Although aniline itself and several substituted anilines are available naturally from coal tar, synthesis from benzene is the major source.

26.2 Basicity of Arylamines

Arylamines, like their aliphatic counterparts, are basic; the lone pair of nonbonding electrons on nitrogen can bond to Lewis acids, yielding an arylammonium salt. The base strength of arylamines is generally lower than that of aliphatic amines, however. Thus, methylammonium ion has pK_a = 10.64, whereas anilinium ion has $pK_a = 4.63$. [*Remember:* The base strength of an amine is inversely related to the acid strength of its corresponding ammonium ion (Section 25.4). A stronger base like methylamine corresponds to a less acidic ammonium ion (higher pK_a) whereas a weaker base like aniline corresponds to a more acidic ammonium ion (lower pK_a).]

Arylamines are less basic than alkylamines because the nitrogen lone-pair electrons are delocalized by orbital overlap with the aromatic ring pi electron system and are less available for bonding. In resonance terms, arylamines are stabilized relative to alkylamines because of the five contributing resonance structures that can be drawn:




ORGANIC
CHEMISTRY
Structure and Function
Fifth Edition
VOLLHARDT
SCHORE

6-2 Nucleophilic Substitution

Haloalkanes contain an electrophilic carbon atom, which may react with *nucleophiles*, substances that contain an unshared electron pair. The nucleophile can be an anion, such as hydroxide ($:\ddot{O}H^-$), or a neutral species, such as ammonia ($:NH_3$). In this process, which we call **nucleophilic substitution**, the reagent attacks the haloalkane and replaces the halide. A great many species are transformed in this way, particularly in solution. The reaction occurs widely in nature and can be controlled effectively even on an industrial scale. Let us see how it works in detail.

Nucleophiles attack electrophilic centers

The nucleophilic substitution of a haloalkane is described by either of two general equations. Recall (Section 2-2) that the curved arrows denote electron-pair movement.

Nucleophilic Substitutions

Negative nucleophile gives neutral product

$$Nu:^- + R\text{—}\ddot{X}: \longrightarrow R\text{—}Nu + :\ddot{X}:^-$$

Nucleophile — Electrophile — Leaving group

Neutral nucleophile gives positively-charged product (as a salt)

$$Nu: + R\text{—}\ddot{X}: \longrightarrow [R\text{—}Nu]^+ + :\ddot{X}:^-$$

Nucleophile — Electrophile — Leaving group

In the first example, a negatively charged nucleophile reacts with a haloalkane to yield a neutral substitution product. In the second example, an uncharged Nu produces a positively charged product. In both cases, the group displaced is the halide ion, $:\ddot{X}:^-$, which is called the **leaving group.** Specific examples of these two types of nucleophilic substitution are shown in Table 6-3. As will be the case in many equations and mechanisms that follow, nucleophiles, electrophiles, and leaving groups are shown here in red, blue, and green, respectively. The general term **substrate** (*substratus*, Latin, to have been subjected) is applied to the organic starting material—in this case, the haloalkane—which is the target of attack by a nucleophile.

Nucleophilic substitution exhibits considerable diversity

Nucleophilic substitution changes the functional group in a molecule. A great many nucleophiles are available to participate in this process; therefore, a wide variety of

6-7 Structure and S_N2 Reactivity: The Leaving Group

The relative facility of S_N2 displacements depends on several factors, including the nature of the leaving group, the reactivity of the nucleophile (which is affected by the choice of reaction solvent), and the structure of the alkyl portion of the substrate. We employ kinetics as our tool to evaluate the degree to which changes in each of these *structural* features affect their *function* in the S_N2 reaction. We begin by examining the leaving group. Subsequent sections will address the nucleophile and the substrate.

Leaving-group ability is a measure of the ease of its displacement

As a general rule, nucleophilic substitution occurs only when the group being displaced, X, is readily able to depart, taking with it the electron pair of the C–X bond. Are there structural features that might allow us to predict, at least qualitatively, whether a leaving group is "good" or "bad"? Not surprisingly, the relative rate at which it can be displaced, its **leaving-group ability,** can be correlated with its capacity to accommodate a negative charge. Remember that a certain amount of negative charge is transferred to the leaving group in the transition state of the reaction (Figure 6-4).

For the halogens, leaving-group ability increases along the series from fluorine to iodine. Thus, iodide is regarded as a "good" leaving group; fluoride, however, is so "poor" that S_N2 reactions of fluoroalkanes are rarely observed.

Leaving-Group Ability

$I^- > Br^- > Cl^- > F^-$

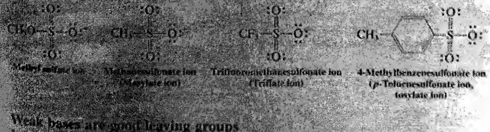
Best — Worst

EXERCISE 6-15

Predict the product of the reaction of 1-chloro-6-iodohexane with one equivalent of sodium methylselenide ($Na^{+}\ ^{-}SeCH_3$).

Halides are not the only groups that can be displaced by nucleophiles in S_N2 reactions. Other examples of good leaving groups are sulfur derivatives of the type $ROSO_3^-$ and RSO_3^-, such as methyl sulfate ion, $CH_3OSO_3^-$, and various sulfonate ions. Alkyl sulfate and sulfonate leaving groups are used so often that trivial names, such as mesylate, triflate, and tosylate, have found their way into the chemical literature.

Sulfate and Sulfonate Leaving Groups



Methyl sulfate ion — Methanesulfonate ion (Mesylate ion) — Trifluoromethanesulfonate ion (Triflate ion) — 4-Methylbenzenesulfonate ion (*p*-Toluenesulfonate ion, tosylate ion)

Weak bases are good leaving groups

Is there some characteristic property that distinguishes good leaving groups from poor ones? Yes. *Leaving-group ability is inversely related to base strength.* Weak bases are best able to accommodate negative charge and are the best leaving groups. Among the halides, iodide is the weakest base and therefore the best leaving group in the series. Sulfates and sulfonates are weak bases as well.

Is there a way to [illegible] weak [illegible] X⁻ is as a base, the [illegible]. Therefore, *good leaving groups are the conjugate* [illegible] applies to the four halides: HI is the weakest of the [illegible]

TABLE 6-7
Relative Rates of Reaction of Various Nucleophiles with Iodomethane in Methanol (Protic Solvent)

Nucleophile	Relative rate
CH_3OH	1
NO_3^-	~32
F^-	500
$CH_3\overset{\overset{O}{\|}}{C}O^-$	20,000
Cl^-	23,500
$(CH_3CH_2)_2S$	219,000
NH_3	316,000
CH_3SCH_3	347,000
N_3^-	603,000
Br^-	617,000
CH_3O^-	1,950,000
CH_3SeCH_3	2,090,000
CN^-	5,010,000
$(CH_3CH_2)_3As$	7,940,000
I^-	26,300,000
HS^-	100,000,000

Nucleophilic st

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ORGANIC CHEMISTRY
Structure and Function

FIFTH EDITION

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